

Seasonal variation in plasma and corpus luteum oestradiol-17 β and progesterone concentrations of the lizard *Cordylus p. polyzonus* (Sauria: Cordylidae)

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Seasonal variation in plasma and corpus luteum oestradiol-17 β and progesterone concentrations of the viviparous lizard *Cordylus p. polyzonus* is described. Steroid concentrations were measured by radioimmunoassay. Plasma oestradiol-17 β concentration increased from basal values in late autumn (May) and peaked in mid-spring (October), during which time females were vitellogenic. Plasma oestradiol-17 β concentration declined to basal values in late spring (November) when females ovulate, remaining low until the onset of the next vitellogenic phase in autumn. Plasma progesterone concentrations of previtellogenic and early vitellogenic females (April–July) were basal, but increased during the later stages of vitellogenesis (August–October). Following ovulation in November, plasma progesterone concentrations were high throughout pregnancy, declining to basal values in April when parturition occurred. Oestradiol-17 β was detectable in freshly formed corpora lutea (corpora haemoragica) during the first month of pregnancy (November), but declined to undetectable values thereafter. Corpus luteum progesterone concentration gradually increased as pregnancy proceeded, reaching a peak two months before birth, but declined thereafter. Progesterone concentration of postpartum degenerating corpora lutea (corpora albicantia) was considerably lower than that of corpora lutea during gestation.

Seisoenale variasie in plasma- en corpus luteum-oestradiol-17 β - en progesteroonkonsentrasies van die lewendbarende akkedis *Cordylus p. polyzonus*, word beskryf. Die steroïdkonsentrasies is bepaal deur radioimmunologie. Plasma-oestradiol-17 β -konsentrasies neem toe vanaf basale waardes gedurende laat herfs (Mei) en bereik 'n piek in middel lente (Oktober) wanneer wyfies vitellogenie is. Plasma-oestradiol-17 β -konsentrasie neem af na basale waardes in laat lente (November) wanneer wyfies ovuleer, en bly laag tot die aanvang van die volgende vitellogenie fase in die herfs. Plasma-progesteroonkonsentrasies van previtellogenie en vroeë vitellogenie wyfies (April–Julie) is basaal, maar neem toe gedurende die later stadia van vitellogenie (Augustus–Oktober). Na ovulasie in November is plasma-progesteroonkonsentrasies hoog en bly so deur swangerskap, gevolg deur 'n afname tot basale waardes in April wanneer geboorte plaasvind. Oestradiol-17 β was bepaalbaar in die nuutgevormde corpora lutea (corpora haemoragica) tydens die eerste maand van swangerskap (November), maar neem af na onbepaalbare vlakke daarna. Corpus luteum-progesteroonkonsentrasies neem geleidelik toe soos swangerskap vorder en bereik 'n piek twee maande voor geboorte, waarna dit afneem. Progesteroonkonsentrasies van degenererende corpora lutea (corpora albicantia) was aansienlik laer as dié van corpora lutea tydens swangerskap.

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Detailed reproductive data are available for both sexes of *Cordylus p. polyzonus* from two geographically separated areas (Van Wyk 1989, 1990; Flemming & Van Wyk 1992; Flemming 1993). These studies indicate little variation in timing of reproductive activities between the two populations, possibly because proximate and ultimate factors timing reproduction in this lizard occur at similar times in the two localities. Numerous factors have been shown to be important in affecting reptilian reproduction, of which external ones (climatic, physical and social) have been studied extensively (Crews & Gans 1992). However, internal factors (both neural and hormonal) that mediate external factors have received little attention (Licht 1984; Whittier & Tokarz 1992). Factors controlling timing and synchrony of reproductive events in *Cordylus p. polyzonus* have not been studied, although there has been some effort to correlate reproductive activities with climatic factors (Van Wyk 1989, 1990; Flemming & Van Wyk 1992; Flemming 1993) and, in males, with circulating sex steroid concentrations (Flemming 1993).

The importance of sex steroids in controlling and synchronizing reptilian reproductive events are well recognized, but there exists a lack of precise information regarding

physiological roles of these steroids (Licht 1984). Data on secretory patterns of sex steroids may be of considerable value in understanding their physiology (Licht 1984). Although information based on indirect estimates of steroid cycles (e.g. from histological or histochemical changes in the gonad or presumptive target organs) are abundant, these methods have serious limitations (see Licht 1982; Licht 1984). Direct measurements of plasma steroid concentrations (Licht 1984) provide more reliable data, but such information is sparse, especially for female viviparous lizards (Licht 1984; Yaron 1985). In viviparous lizards, oestrogens appear to regulate vitellogenesis and female sexual reproductive behaviour as in oviparous ones, and vertebrates in general (Licht 1984). The role of progestins on the other hand, especially in maintaining pregnancy, is not clear (Yaron 1985). Furthermore, data on the source of circulating progestins are conflicting (Guillette 1985; Yaron 1985).

In this study seasonal variation in plasma and corpus luteum oestradiol-17 β and progesterone concentrations of the viviparous lizard *Cordylus p. polyzonus* was investigated and correlated with aspects of the ovarian cycle previously reported by Flemming & Van Wyk (1992).

Materials and Methods

Autopsy procedure

Specimens were collected monthly over the period of one year (February 1986–January 1987) at Saldanha Bay (33°00'S / 17°56'E) in the south-western Cape Province, South Africa. To ensure sexual maturity only females with a snout-vent length of > 95 mm were used in this study (see Flemming & Van Wyk 1992). Females were anaesthetized with diethyl ether within 24 h of capture. Blood samples (0,40–1,20 ml) were obtained by decapitation, centrifuged and the plasma stored at –20°C until further analysis. During autopsy of gravid females, corpora lutea were excised from ovaries, and those collected in the same month were pooled, weighed to the nearest 0,1 mg and stored at –20°C. The same procedure was followed for degenerating corpora lutea (corpora albicantia) during the postpartum months April–June, but these were pooled in a single sample. Just prior to steroid content determination, luteal samples were homogenized in 0,9% saline.

Radioimmunoassays

Steroid concentrations were determined by radioimmunoassay at the Department of Chemical Pathology, Medical School, University of Cape Town, South Africa. Antisera used were developed and kindly supplied by M.S. Hendricks from this department.

Oestradiol-17 β concentrations were determined from duplicate aliquants (0,20 ml) of plasma or corpus luteum homogenate, using a procedure similar to that of Bennet & Jarvis (1988). Tritiated oestradiol (TRK587, Radiochemical Center, Amersham, UK) was used as tracer and an antiserum raised in rabbits against oestradiol-17 β -carboxymethyl-oxime-bovine serum albumin according to the method of Dean, Exley & Johnson (1971). The antiserum showed very low cross reactivity with oestrone (0,05%) and less than 0,01% with other naturally occurring steroids.

Progesterone concentration measurements were performed on duplicate aliquants (0,20 ml) of plasma or corpus luteum homogenate, using a procedure similar to that of Bernard, Bojarski & Millar (1991). Tritiated progesterone (TRK413, Radiochemical Center, Amersham, UK) was used as tracer, and an antiserum raised against progesterone-21-bovine serum albumin according to the method of Erlanger, Borek, Beiser & Lieberman (1959). The antiserum showed low cross reactivity with 5 α pregnanedione (3,0%), and less than 0,1% with other naturally occurring steroids.

Extraction efficiency as suggested by the recovery of the tracer added to plasma/corpus luteum aliquants was 95–98% for the oestradiol assay and 89–95% for the progesterone assay. The intra-assay coefficient of variance (determined by an assay of replicate aliquots of plasma controls) was 7,2% for oestradiol-17 β and 6,4% for progesterone. The between-assay-variances for the same samples were 9,1% for oestradiol-17 β and 9,3% for progesterone. Sensitivity of the assays was 156 pmol/l. Serial dilutions of plasma samples gave displacement curves parallel to the standard curve.

Statistical analyses

One-way analysis of variance (ANOVA) was used to analyse seasonal variation in steroid concentrations, followed

by Tukey's multiple range test to determine significant differences in monthly samples (Sokal & Rohlf 1981). A Pearson's product-moment correlation coefficient test was performed to determine if any correlation existed between ovarian follicle diameter (previously reported in Flemming & Van Wyk 1992) and plasma oestradiol-17 β and progesterone concentrations; $p < 0,05$ was considered as significant. All means are followed by standard error of the mean.

Results

The ovarian cycle

Summary diagrams indicating seasonal variation in follicle diameter and reproductive condition of female *Cordylus p. polyzonus* are presented in Figure 1a and b respectively. Data are from Flemming & Van Wyk (1992).

Plasma oestradiol-17 β concentrations

Variation in plasma oestradiol-17 β concentration showed a significant annual trend (ANOVA; $F_{11,64} = 243,9$; $p < 0,05$; Figure 2a). Plasma oestradiol-17 β concentrations increased progressively from May to reach a peak in October. This period of elevated plasma oestradiol-17 β concentration coincided with increasing ovarian follicle diameter and vitellogenic activity (Figure 1a,b). Oestradiol-17 β concentrations declined to basal values during ovulation (November), persisting through pregnancy until the onset of the next vitellogenic phase.

Plasma oestradiol-17 β concentrations were significantly and positively correlated with seasonal changes in ovarian follicle diameter ($r = 0,72$; $p < 0,05$).

Plasma progesterone concentrations

Plasma progesterone concentrations exhibited considerable individual variation, but showed a significant annual trend

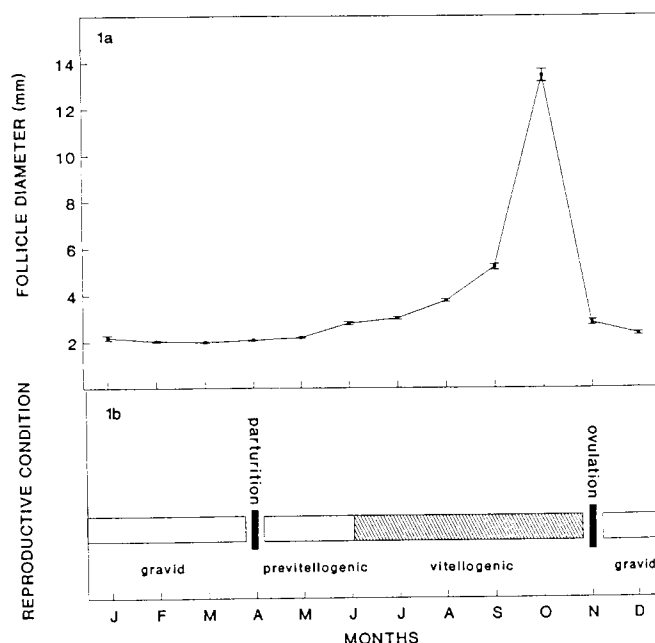


Figure 1 (a) Seasonal variation in ovarian follicle diameters (mean \pm 1 SE) and (b) changes in reproductive condition of female *Cordylus polyzonus*. Data are from Flemming & Van Wyk (1992).

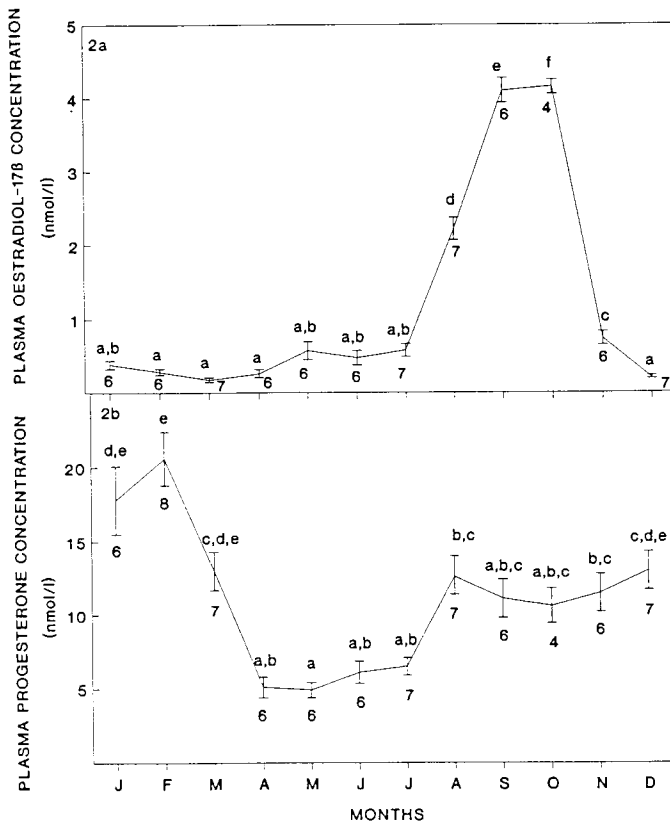


Figure 2 Variation in plasma concentrations of (a) oestradiol-17 β and (b) progesterone during the reproductive cycle of female *Cordylus polyzonus*. Values are presented as mean \pm 1SE. Numbers below values indicate sample sizes. Values with different superscripts are significantly different from each other (Tukey's Multiple Range Test; $p < 0,05$).

($F_{11,64} = 13,6$; $p < 0,05$; Figure 2b). Plasma progesterone concentrations were basal in previtellogenic and early vitellogenic females (April–July). Plasma progesterone concentrations increased slightly during later vitellogenesis (August–October), but did not differ significantly from those of previtellogenic or early vitellogenic females. Following ovulation in November, plasma progesterone concentrations were significantly elevated in gravid females compared to those of previtellogenic and early vitellogenic ones. Plasma progesterone concentrations declined during the last month of pregnancy and reached basal values in April when parturition occurred.

There was no significant correlation between plasma progesterone concentrations and seasonal changes in ovarian follicle diameters ($r = 0,41$; $p > 0,01$).

Corpus luteum steroid concentrations

Corpus luteum oestradiol-17 β and progesterone concentrations are presented in Figure 3. In freshly formed corpora lutea (November) oestradiol-17 β concentrations measured 49 ng/g, but declined to undetectable values during the following months of pregnancy. Corpus luteum progesterone concentrations were relatively low in November, but increased progressively to reach a peak in March. Progesterone concentrations (April–May) of degenerating corpora lutea (corpora albicantia) after parturition were considerably lower than during pregnancy.

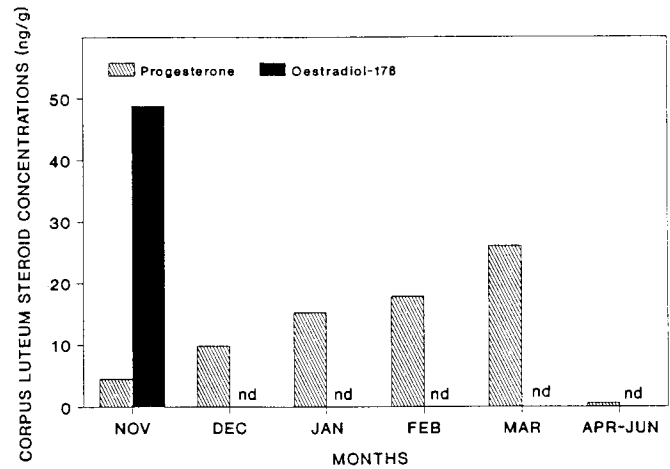


Figure 3 Variation in the corpus luteum oestradiol-17 β and progesterone concentrations in female *Cordylus p. polyzonus*.

Discussion

The reptilian ovary secretes a variety of steroid hormones (oestrogens, androgens and progestins), of which the physiological actions of oestrogens have probably been best studied to date (Licht 1984). Like in other tetrapods, oestrogens in reptiles are important in regulating ovarian growth, vitellin production by the liver, for hypertrophy and biochemical activity of the oviducts, and for female sexual behaviour (Licht 1984). Oestradiol is considered the major hormone controlling vitellogenesis in reptiles and the involvement, if any, of other steroids is still unclear (Licht 1984; Guraya 1989). In *Cordylus p. polyzonus*, high oestradiol-17 β concentrations coincided with vitellogenesis and increasing ovarian follicle diameters. During this time oviducts, fat bodies and livers also show weight increases in *Cordylus p. polyzonus* (Flemming & Van Wyk 1992). Similar results reported for other reptiles (Lance & Callard 1978; Callard & Ho 1980; Licht 1984) suggest regulation of vitellogenesis by oestrogens in *Cordylus p. polyzonus*.

Although progesterone is believed to be primarily associated with ovulatory and post-ovulatory events, it may play an important role in the preovulatory phase of vitellogenic activity (Licht 1984). Some of the proposed functions of progesterone during the preovulatory phase include a synergism with oestrogens in stimulating oviduct hypertrophy (Jones & Guillette 1982), inhibition and termination of yolk deposition (Callard & Doolittle 1973; Yaron & Widzer 1978), as well as initiation of ovulation or triggering of ovulation itself (Licht 1982; McPherson, Boots, MacGregor & Marion 1982). There was evidence of an increase in plasma progesterone concentrations during late vitellogenesis in female *Cordylus p. polyzonus*, but concentrations during this time did not differ significantly from that of previtellogenic and early vitellogenic females. Significant increases in progesterone concentrations during vitellogenesis were reported for some reptiles (Callard, Lance, Salhanick & Barad 1978; Bona-Gallo, Licht, MacKenzie & Lofts 1980; Ho, Kleis, McPherson, Heisermann & Callard 1982; McPherson *et al.* 1982; Van Wyk 1984). In others, however, plasma progesterone concentrations are low during follicular growth (Bourne & Seamark 1972;

Veith 1974; Lance & Callard 1978). It has been illustrated that in many reptiles, progesterone does not act synergistically with oestrogens and has an inhibitory effect on the growth of ovarian follicles and vitellogenesis (Callard & Doolittle 1973; Callard & Klotz 1973; Klicka & Mahmoud 1973; Veith 1974; Yaron & Widzer 1978; Licht 1982). It is not clear from the seasonal circulatory pattern whether progesterone has a stimulatory or inhibitory effect on vitellogenesis in *Cordylus p. polyzonus*. As the physiological actions of progesterone have not been studied in *Cordylus p. polyzonus*, the role of progesterone in preovulatory events of this species is unknown.

In virtually all reptiles studied, including viviparous ones, progesterone concentrations rose dramatically after ovulation, possibly owing to the formation of secretory corpora lutea (Licht 1984; Yaron 1985). The post-ovulatory function of progesterone in viviparous reptiles appears to include preparation and maintenance of oviducts (Yaron 1972; Browning 1973), inhibition of oviductal contraction (Callard & Hirsch 1976) and inhibition of further ovarian development until the young are born (Yaron 1985). In *Cordylus p. polyzonus* plasma progesterone concentrations were elevated and reached a maximum during pregnancy, suggesting that this hormone plays an important role in the maintenance of pregnancy.

Plasma progesterone concentrations may vary during pregnancy and patterns differ among reptilian species (Yaron 1985; Xavier 1987). In the lizard *Sceloporus cyanogenys* (Callard, Bayne & McConnell 1972), progesterone concentrations were comparable during early and late pregnancy. In the chameleon *Bradypodion pumilum* (Veith 1974), plasma progesterone dropped by about 50% from early to mid-pregnancy. In the garter snake *Thamnophis elegans* (Highfill & Mead 1975), plasma progesterone increased between the first and second trimester of gestation, but declined during the last trimester. Similar changes were recorded for the snakes *Nerodia sipedon* and *Thamnophis elegans* (Chan, Ziegel & Callard 1973). In *Cordylus p. polyzonus* plasma progesterone concentrations progressively increased as pregnancy proceeded, reaching highest values two months prepartum and declined during the last month of pregnancy.

Preovulatory plasma progesterone concentrations possibly originate from the ovarian follicle and/or the adrenal gland (Guillette, Spielvogel & Moore 1981; Dauphin-Villemant, Le Boulenger, Xavier & Vaudry 1990; Naulleau & Fleury 1990). The source of preovulatory plasma progesterone in *Cordylus p. polyzonus* is, however, unknown.

The steroid-secreting capacity of the reptilian corpus luteum is well known (Klicka & Mahmoud 1973; Chan & Callard 1974; Callard, McChesney, Scanes & Callard 1976) and in viviparous reptiles is believed to be the major source of circulating progesterone during the post-ovulatory phase (Yaron 1985). Other suggested sources of circulating progesterone in post-ovulatory females are the adrenals, ovaries (ovarian tissue and atretic follicles), and the choriollantoic placenta (Guillette & Fox 1985; Dauphin-Villemant *et al.* 1990; Naulleau & Fleury 1990). The freshly formed corpus luteum in *Cordylus p. polyzonus* still had detectable concentrations of oestradiol-17 β , possibly resulting from the oestrogen-secreting capacity of the preovulatory follicle.

However, oestradiol-17 β concentrations in corpora lutea of *Cordylus p. polyzonus* became undetectable during the following months of pregnancy. Progesterone concentration of corpus luteum tissue has previously been determined in three reptilian species. In the chameleon *Bradypodion pumilum* corpus luteum tissue contained 56,8 μ g/g progesterone (Veith 1974). Corpus luteum progesterone concentration in the lizard *Uromastix hardwicki* was 531,0 \pm 73,4 ng/g (Arslan, Zaidi, Lobo, Zaidi & Qasi 1978) and that of the garter snake *Thamnophis elegans* ranged from 1,5–26 ng/g (Highfill & Mead 1975). In *Cordylus p. polyzonus*, corpus luteum progesterone concentrations (4,8–26,2 ng/g) were lower than those reported for *Bradypodion pumilum* and *Uromastix hardwicki*, but comparable to those of *Thamnophis elegans*. It needs to be experimentally illustrated, however, whether the reported plasma progesterone concentrations during gestation in *Cordylus p. polyzonus* result primarily from the corpus luteum and to what extent gestation depends on this structure. As the corpus luteum is regressed or absent in the postpartum ovary, this structure is unlikely to contribute to plasma progesterone concentrations observed during the previtellogenic or vitellogenic phase in *Cordylus p. polyzonus*.

The timing of luteal regression differs among viviparous lizards (Guillette 1985). *Cordylus p. polyzonus* corresponds to those having actively secreting corpora lutea until late pregnancy (Guillette 1985). However, some viviparous lizards exhibit luteal regression during mid-pregnancy, or even earlier in *Sceloporus jarrovi* (Guillette *et al.* 1981; Guillette 1985).

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